

CLAIMS:

1. A method for characterising an analyte by matrix assisted laser desorption ionisation (MALDI) mass spectrometry, which method comprises:
 - (a) labelling the analyte with a light-absorbing label that absorbs light at a pre-determined frequency, to form a labelled analyte;
 - (b) embedding the labelled analyte in a matrix formed from at least one compound that absorbs light, to form an embedded labelled analyte;
 - (c) desorbing the embedded labelled analyte by exposing it to light having the pre-determined frequency, to form a desorbed analyte; and
 - (d) detecting the desorbed analyte by mass spectrometry to characterise the analyte.
2. A method according to claim 1, wherein the desorbed analyte is directly detected by mass spectrometry.
3. A method according to claim 1, in which the desorbed analyte is indirectly detected by mass spectrometry, wherein the analyte is additionally labelled with a mass label relatable to the analyte, and wherein the mass label is cleaved from the desorbed analyte and detected by mass spectrometry to characterise the analyte.
4. A method according to any preceding claim, wherein the light to which the embedded labelled analyte is exposed is laser light.
5. A method according to any preceding claim, wherein the compound forming the matrix absorbs light at the same frequency as the light-absorbing label.
6. A method according to any preceding claim, wherein the matrix and the light-absorbing label are formed from the same compound.

7. A method according to any preceding claim, wherein the matrix is a solid matrix or a liquid matrix.
8. A method according to claim 7, wherein the matrix is a liquid matrix comprising nitrobenzyl alcohol.
9. A method according to any preceding claim, wherein the matrix comprises an acid matrix or a basic matrix.
10. A method according to claim 9, wherein the matrix comprises a compound selected from 3-hydroxypicolinic acid, 2,5-dihydroxybenzoic acid and 4-hydroxy-alpha-cyanocinnamic acid.
11. A method according to any preceding claim, wherein the light-absorbing label is formed from a dye.
12. A method according to claim 11, wherein the dye is a non-fluorescent dye.
13. A method according to claim 11 or claim 12, wherein the dye comprises 4-dimethylaminoazobenzene-4'-sulphonyl chloride (DABSYL chloride), 3-hydroxypicolinic acid, 2,5-dihydroxybenzoic acid and 4-hydroxy-alpha-cyanocinnamic acid.
14. A method according to any preceding claim, wherein the analyte comprises one or more compounds selected from a protein, a polypeptide, a peptide, a peptide fragment and an amino acid.
15. A method for characterising a polypeptide, which method comprises the steps of:
 - (a) optionally reducing cysteine disulphide bridges in the polypeptide to form free thiols, and capping the free thiols;

- (b) cleaving the polypeptide with a sequence specific cleavage reagent to form peptide fragments;
- (c) optionally deactivating the cleavage reagent;
- (d) capping one or more ϵ -amino groups that are present with a lysine reactive agent;
- (e) analysing peptide fragments according to a method as defined in any of claims 1-14 to form a mass fingerprint for the polypeptide; and
- (f) determining the identity of the polypeptide from the mass fingerprint.

16. A method for characterising a population of polypeptides, which method comprises the steps of:

- (h) optionally reducing cysteine disulphide bridges in one or more polypeptides to form free thiols, and capping the free thiols;
- (i) separating one or more polypeptides from the population;
- (j) cleaving one or more polypeptides with a sequence specific cleavage reagent to form peptide fragments;
- (k) optionally deactivating the cleavage reagent;
- (l) capping one or more ϵ -amino groups that are present with a lysine reactive agent;
- (m) analysing peptide fragments according to a method as defined in any of claims 1-14 to form a mass fingerprint for one or more polypeptides; and
- (n) determining the identity of one or more polypeptides from the mass fingerprint.

17. A method for comparing a plurality of samples, each sample comprising one or more polypeptides, which method comprises the steps of:

- (h) optionally reducing cysteine disulphide bridges and capping the free thiols in one or more polypeptides from the samples;
- (i) separating one or more polypeptides from each of the samples;

- (j) cleaving the polypeptides with a sequence specific cleavage reagent to form peptide fragments;
- (k) optionally deactivating the cleavage reagent;
- (l) capping one or more ϵ -amino groups that are present with a lysine reactive agent;
- (m) analysing peptide fragments according to a method as defined in any of claims 1-14 to form a mass fingerprint for one or more polypeptides from the samples; and
- (n) determining the identity of one or more polypeptides in the samples from one or more mass fingerprints.

18. A method according to any of claims 15-17, wherein the lysine-reactive agent is a labelled lysine-reactive agent.

19. A method according to claim 17, for comparing a plurality of samples, each sample comprising one or more polypeptides, which method comprises the steps of:

- (a) optionally reducing cysteine disulphide bridges and capping the free thiols in one or more polypeptides from the samples;
- (b) capping one or more ϵ -amino groups that are present in each sample with a labelled lysine reactive agent;
- (c) pooling the samples;
- (d) separating one or more polypeptides from the pooled samples;
- (e) cleaving the polypeptides with a sequence specific cleavage reagent to form peptide fragments;
- (f) optionally deactivating the cleavage reagent;
- (g) analysing peptide fragments according to a method as defined in any of claims 1-14 to form a mass fingerprint for one or more polypeptides from the samples; and
- (h) determining the identity of one or more polypeptides in the samples from one or more mass fingerprints.

wherein the same label is employed for polypeptides or peptides from the same sample, and different labels are employed for polypeptides or peptides from different samples, such that the sample from which a polypeptide or peptide originates can be determined from its label.

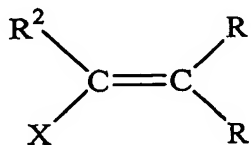
20. A method according to any of claims 15-19, wherein the sequence specific cleavage agent cleaves the one or more polypeptides on the C-terminal side of a lysine residue.

21. A method according to any of claims 15-20, wherein the specific cleavage reagent comprises Lys-C or Trypsin.

22. A method according to any of claims 15-21, wherein the peptide fragments having capped ϵ -amino groups are removed by affinity capture, and wherein the lysine reactive agent comprises biotin.

23. A method according to any of claims 15-22, wherein the lysine reactive agent comprises a hindered Michael reagent.

24. A method according to claim 23, wherein the hindered Michael agent comprises a compound having the following structure:



wherein X is an electron withdrawing group that is capable of stabilising a negative charge; the R groups independently comprise a hydrogen, a halogen, an alkyl, an aryl, or an aromatic group with the proviso that at least one of the R groups comprises a sterically hindering group; and the group R^2 comprises a hydrogen, a halogen, a hydrocarbon

group, an electron withdrawing group and/or a linker capable of attachment to an affinity capture functionality or a solid phase support.

25. A labelled analyte compound, which compound has the following structure:



wherein D comprises a light absorbing label, M comprises a mass modifier, L comprises a linker and A comprises an analyte.

26. A compound according to claim 25, wherein D comprises a non-fluorescent dye.

27. A compound according to claim 26, wherein D comprises a cinnamic acid derivative, a nicotinic acid derivative, a picolinic acid derivative, a hydroxybenzoic acid derivative, a methoxybenzoic acid derivative or a sinapinic acid derivative.

28. A compound according to claim 26 or claim 27, wherein the non-fluorescent dye comprises a compound selected from 4-dimethylaminoazobenzene-4'-sulphonyl chloride (DABSYL chloride), 3-hydroxypicolinic acid, 2,5-dihydroxybenzoic acid and 4-hydroxy- α -cyanocinnamic acid.

29. A compound according to any of claims 25-28, wherein M is selected from a compound formed from an aryl ether, and an oligomer formed from 2 or more aryl ether units.

30. A compound according to any of claims 25-28, wherein the linker comprises a group selected from $-\text{CR}_2-\text{CH}_2-\text{SO}_2-$, $-\text{N}(\text{CR}_2-\text{CH}_2-\text{SO}_2-)_2$, $-\text{NH}-\text{CR}_2-\text{CH}_2-\text{SO}_2-$, $-\text{CO}-\text{NH}-$, $-\text{CO}-\text{O}-$, $-\text{NH}-\text{CO}-\text{NH}-$, $-\text{NH}-\text{CS}-\text{NH}-$, $-\text{CH}_2-\text{NH}-$, $-\text{SO}_2-\text{NH}-$, $-\text{NH}-\text{CH}_2-\text{CH}_2-$ and $-\text{OP}(=\text{O})(\text{O})\text{O}-$.

31. A compound according to any of claims 25-30, wherein A is selected from a protein, a polypeptide, a peptide, a peptide fragment and an amino acid.

32. A compound for labelling an analyte, which compound has the following structure:



wherein M is a mass marker comprising a cinnamic acid derivative, a nicotinic acid derivative, a picolinic acid derivative, a hydroxybenzoic acid derivative, a methoxybenzoic acid derivative or a sinapinic acid derivative, L comprises a linker and R comprises a reactive functionality for attaching the compound to an analyte.

33. A compound according to claim 32, wherein M comprises a non-fluorescent dye compound selected from 4-dimethylaminoazobenzene-4'-sulphonyl chloride (DABSYL chloride), 3-hydroxypicolinic acid, 2,5-dihydroxybenzoic acid and 4-hydroxy- α -cyanocinnamic acid.

34. A compound according to claim 32 or claim 33, wherein M further comprises a compound formed from an aryl ether, or an oligomer formed from 2 or more aryl ether units.

35. A compound according to any of claims 32-34, wherein the linker comprises a group selected from $-CR_2-CH_2-SO_2-$, $-N(CR_2-CH_2-SO_2-)_2$, $-NH-CR_2-CH_2-SO_2-$, $-CO-NH-$, $-CO-O-$, $-NH-CO-NH-$, $-NH-CS-NH-$, $-CH_2-NH-$, $-SO_2-NH-$, $-NH-CH_2-CH_2-$, $-OP(=O)(O)O-$ and an amide linkage through the acid groups of M.

36. A compound according to any of claims 32-35, wherein R is for attaching the compound to a protein, a polypeptide, a peptide, a peptide fragment or an amino acid.

37. A compound according to any of claims 32-36, wherein R comprises an ester group, an acid anhydride group, an acid halide group such as an acid chloride, an N-hydroxysuccinamide group, a pentafluorophenyl ester group, a maleimide group, an alkenyl sulphone group, or an iodoacetamide group.

38. A compound according to any of claims 25-37, which compound further comprises an affinity ligand.

39. A compound according to claim 38, wherein the affinity ligand comprises biotin.

40. A compound according to any of claims 25-39, which compound further comprises an ionisable moiety.

41. A compound according to claim 40, wherein the ionisable moiety is selected from a tertiary amino group, a guanidino group and a sulphonic acid group.

42. A compound according to any of claims 25-41, which compound comprises a cinnamic acid functionality.

43. An array of two or more compounds for labelling an analyte, wherein the compounds in the array are compounds as defined in any of claims 32-42 and wherein each compound in the array differs in mass from all other compounds in the array.

44. An array of compounds according to claim 43, wherein the difference in mass of each of the compounds in the array is achieved by isotopic substitution.

45. A kit for characterising an analyte by matrix assisted laser desorption ionisation (MALDI) mass spectrometry, which kit comprises:

- (a) one or more light absorbing labels having a reactive functionality for attaching the labels to an analyte; and

- (b) a compound for forming a matrix, which compound absorbs light at the same frequency as the light-absorbing label.

46. A kit according to claim 45, wherein the component (a) comprises a compound, or an array of compounds, as defined in any of claims 32-44.

47. A kit for characterising an analyte by matrix assisted laser desorption ionisation (MALDI) mass spectrometry, which kit comprises:

- (a) a compound, or an array of compounds, as defined in any of claims 32-44;
- (b) an ion exchange resin.

48. A kit according to claim 47, wherein the compound or array of compounds comprises an ionisable moiety that forms a positive charge, and wherein the ion exchange resin comprises a cation exchange resin.

49. A kit according to claim 47, wherein the compound or array of compounds comprises an ionisable moiety that forms a negative charge, and wherein the ion exchange resin comprises an anion exchange resin.